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EFFECTS OF TWO INSECT GROWTH REGULATORS (HYDROPRENE AND R-20458) ON THE FOLLICULAR EPITHELIUM AND THE OOCYTES OF THE RICE WEEVIL, *SITOPHILUS ORYZAE* (L.) (COLEOPTERA: CURCULIONIDAE)

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Abstract.—Effects of two insect growth regulators (IGRs) (hydroprene and R-20458) on the follicular epithelium and the oocytes of the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) were studied. Comparative histological studies of the treated and untreated ovarioles revealed that there were no apparent differences in the development and structure of the oocytes in the germarium and in the anterior part of the vitellarium. The IGRs, however, affected the penultimate oocytes and perhaps the basal oocytes, because the latter ovulated prematurely. Treated penultimate oocytes were atrophied, because they lacked yolk and karyospheres; in addition, follicular epithelium was not retracted from the surface of the oolemma. Untreated penultimate oocytes on the other hand, were larger due to the accumulation of yolk and they had karyospheres; the follicular epithelium was retracted from the egg membrane, leaving a space that had materials, which possibly are blood proteins. A fine brush-like border (microvilli) was observed on the side of the oolemma facing the follicular epithelium.

Introduction

Laboratory evaluation of IGRs as protectants against pests of stored products have shown considerable promise (Metwally et al. 1972; Bhatnager-Thomas 1973; Strong and Diekman 1973; Williams and Amos 1974; McGregor and Kramer 1975; Loschiavo 1976; and Amos and Williams 1977). It has also been demonstrated that treatment of certain species of insects with IGRs induces permanent ovarial abnormalities that lead to female sterility (Metwally and Landa 1972; Metwally et al. 1972; Rohdendorf and Sehnal 1973; Lanzrein 1974; Patterson 1974; Das and Gupta 1977; Masner et al. 1979; and Deb and Chakravorty 1981).

The purpose of this work was to find out whether hydroprene and R-20458 will induce ovarial abnormalities that might cause sterility in the female weevil.

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Materials and Methods

Several IGRs were screened for their effects, and hydroprene (ethyl(2E,4E)-3,7,11-trimethyl-2,4-dodecadienoate) and R-20458 (6,7-epoxy-3,7-dimethyl-1-(*p*-ethylphenoxy)-2-octene) were selected because they were more effective than others against *S. oryzae*. Of these two, hydroprene was more effective (Mkhize and Gupta 1980; Gupta and Mkhize 1982).

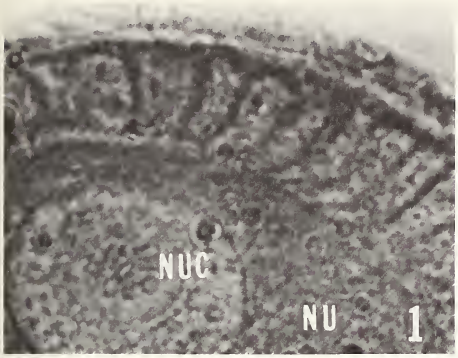
Ovaries of rice weevils, which emerged from either IGR-treated wheat (see Gupta and Mkhize 1982) or topical applications, were removed in saline solution and fixed in two successive changes of Bouin's fluid. The tissues were then dehydrated in ethanol series, cleared in xylene and embedded in paraffin wax. Longitudinal sections, each 5-μm thick, were stained in hematoxylin and eosin and mounted in permount. Histology of treated and untreated ovaries was compared in order to discover morphological abnormalities induced by IGR treatment.

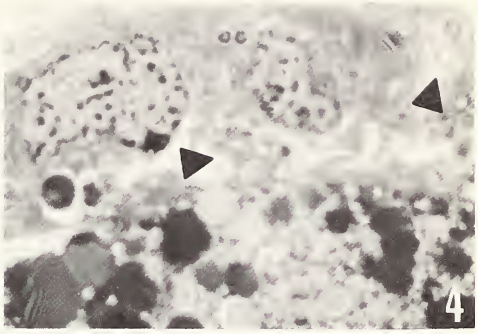
For making ultrathin sections, treated and untreated ovaries were separately dissected in physiological saline solution and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for four hr. After rinsing the ovaries in a buffer, they were placed in 1% solution of osmium tetroxide for two hr. The ovaries were again rinsed in a buffer and then dehydrated in acetone as follows: they were placed in 30%, 70%, and 95% acetone for 20 min in each concentration; then in three changes of 100% acetone for 20 min/change. They were embedded in Epon 812. One μm-thick cross sections were made by Sorvall MT-2 ultramicrotome. The sections were stained for one min, and mounted in immersion oil. The sections were then sealed with permount. Light micrographs of cross sections of both treated and untreated oocytes were made and compared in order to observe abnormalities owing to IGR treatment.

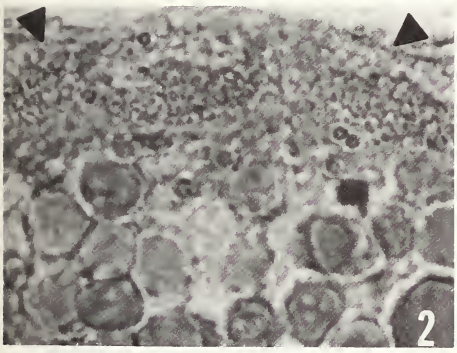
Results

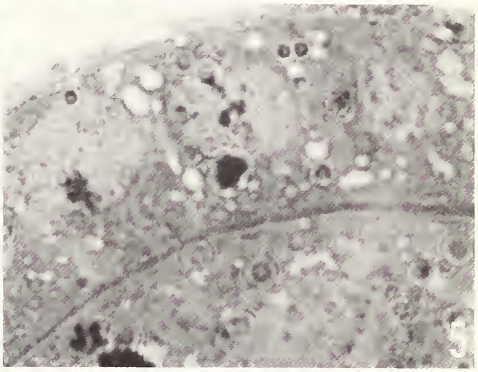
Examination of treated and untreated ovarioles revealed that the germarium and the anterior region of the vitellarium showed no apparent structural or developmental differences between the oocytes in the treated and untreated ovarioles. Differences, however, were observed in the penultimate oocytes and the follicular epithelium around them. In the anterior region of the vitellarium, the cells of the follicular epithelium were columnal in shape and had elongated nuclei (Fig. 1). The cell boundaries were distinct and the cytoplasm appeared strongly basophilic, probably indicative of synthetic processes taking place in these cells.

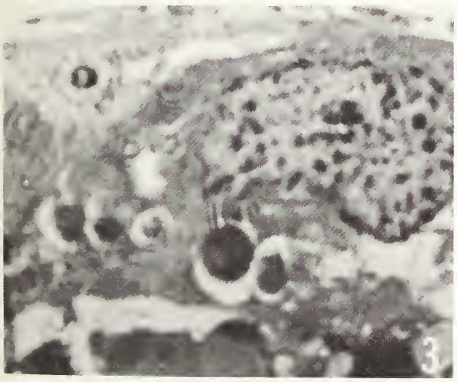
The follicular epithelium around untreated penultimate oocytes had cells that appeared slightly flattened, with small, elongated nuclei, indicating that these cells were in a transitional stage from cuboidal to squamous type of cells (Fig. 2). The epithelium also showed mitotic divisions (Fig. 3) and the nuclear chromatin material seemed to be evenly dispersed. Figure 4 shows

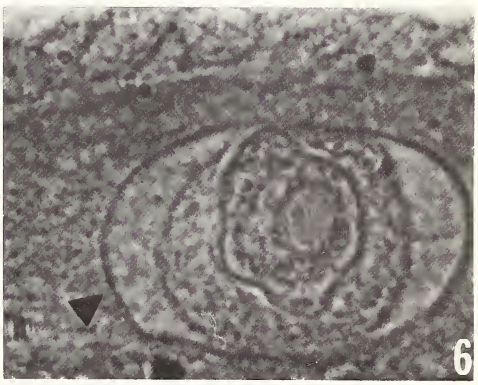












Figs. 1–6. 1. X-section of treated, young oocyte, showing columnar epithelium. NU =nucleus of oocyte; NUC = Nucleolus. ×3,500. 2, 3. X-sections of untreated penultimate oocytes, showing squamous follicular epithelium (arrow head) (Fig. 2, ×3,040) and mitotic figure (Fig. 3, ×2,945). 4. X-section of an untreated penultimate oocyte engaged in vitellogenesis. Note space between oolemma and follicular epithelium and inter-cellular space in the follicular epithelium (arrow head). ×2,945. 5. X-section of a treated penultimate oocyte. Note absence of intercellular space in the follicular epithelium and between the latter and the oolemma. ×3,040. 6. X-section of an untreated oocyte engaged in vitellogenesis, showing a germinal vesicle (arrow head). ×3,420.

the follicular epithelium of an untreated penultimatic oocyte actively engaged in vitellogenesis. This epithelium was retracted from the surface of the egg membrane (oolemma) creating a space between the two interfaces. In addition, there were intercellular spaces in the epithelium itself. These spaces contained material that was less granular than that in the oocyte. Focusing up and down over the space between the follicular epithelium and the oolemma sometimes revealed brush-like border on the oolemma. Retraction of the follicular epithelium from the oolemma enables hemolymph proteins to pass into the oocyte, supposedly by pinocytosis. By contrast, the follicular epithelium in a treated penultimate oocyte was not retracted from the oolemma (Fig. 5) and no intercellular spaces were present. The follicular cells were roundish with spherical nuclei. No mitosis was observed.

Untreated young oocytes in the anterior region of the vitellarium in both treated and untreated oocytes had centrally located nuclei (Fig. 1), with evenly distributed chromatin material. Mature untreated oocytes engaged in vitellogenesis showed germinal vesicle (=the enlarged oocyte nucleus due to accumulation of vesicular fluid) (Fig. 6) and karyosphere (the clumped chromatin material of the nucleus), both of which were not observed in treated oocytes.

Discussion

The follicular epithelium plays an important role in vitellogenesis. The synthesis of enzymes necessary for the uptake of nutrients from the hemolymph (De Loof and Lagasse 1970), proteins (King and Burnett 1959; Zalokar 1960; Beir 1962, 1963b) and of adsorbents that link blood proteins to the outer surface of the oocyte (Roth and Porter 1964; Anderson and Telfer 1970; Anderson 1971) is reported to occur in the follicular epithelium. The facts that the follicular epithelium in the IGR-treated ovarioles were not retracted from the oolemma, showed no mitosis, and the cells were roundish, suggest abnormalities that prevent successful completion of vitellogenesis. Retraction of the follicular epithelium from the oolemma enables hemolymph proteins to pass into the oocyte by pinocytosis (Beir 1962, 1963a; Kessel and Beams 1963; Telfer and Melius 1963; Roth and Porter 1964; Stay 1965; Hopkins and King 1966; De Loof and Lagasse 1970). Clearly, the IGRs in this study blocked retraction of the oolemma and thus vitellogenesis.

In most cases, during vitellogenesis the development of the oocyte nucleus is arrested at meiotic prophase and that just before vitellogensis takes place, both the nucleus and the cytoplasm become very basophilic and rich in RNA (Bonhag 1958; Mahowald 1972; De Robertes et al. 1975; Ambrose and Easty 1978). According to Schlottman and Bonhag (1956) and Gupta and Riley (1967) at about this time, the nucleus attains maximal size due to accumulation of vesicular fluid and is known as germinal vesicle, whose chromatin material is clumped and is called karyosphere (Chandley 1966). The

absence of the germinal vesicle and the karyosphere in the treated oocyte confirms the disruption of vitellogenesis. As a result of the abnormalities discussed in the foregoing, the ovarioles in the IGR-treated weevils remain atrophied, and consequently no F_1 progeny is produced.

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